REMARKS

With entry of the amendments, claims 1-3 and 5-15 are pending in the application. In the Final Office Action, mailed May 9, 2002, the Examiner indicated that the following objections or rejections were withdrawn: objection to the declaration; the rejection of claims 12, 14, and 15 under 35 U.S.C. 101; the rejection of claims 5, 6, and 8 under 35 U.S.C. 112, second paragraph; and the rejection of claim 14 under 35 U.S.C. 102(b) as being anticipated by Barnes *et al.* Claims 1 and 15 are rejected under 35 U.S.C. 112, first paragraph; claims 12, 14 and 15 stand rejected under 35 U.S.C. 102(b); and claims 1-15 stand rejected under 35 U.S.C. 103(a).

Applicants have amended claims 1 and 15, and cancelled claim 4. The amendments are fully supported by the specification, introduce no new matter, and place the claims in better form for consideration on appeal.

In view of the amendments above and the arguments below, Applicants respectfully request reconsideration on the merits of the application and allowance of the claims.

Rejection under 35 U.S.C. 112, first paragraph

Claims 1 and 15 stand rejected under 35 U.S.C. 112, first paragraph. The Examiner asserted that the amendment to include the recitation of "a mammalian species" introduced new matter. Applicants have amended claim 1 to limit the method to one using a bovine recipient oocyte. Applicants have amended claim 15 to limit the embryo to one comprising cytoplasm and cell membrane from a bovine oocyte. Both amendments are fully supported by the specification. In view of the amendment, Applicants request withdrawal of the rejection under 35 U.S.C. 112, first paragraph.

Rejections under 35 U.S.C. 102(b)

Claims 12 and 15 stand rejected as being anticipated by Barnes *et al.* as evidenced by Telford *et al.* (Mol. Reprod. Dev., 26:90-100, 1990). The rejection of claim 14 on the same grounds has been withdrawn. The Barnes *et al.* publication is identified only as the "Mol. Reprod. Dev. 1992 IDS reference." Applicants note that the only Barnes IDS reference is Barnes and First (Mol. Reprod. Dev., 29:117-123, 1991), and assume that it is this publication to which the Examiner intended to refer.

The Examiner asserted that claim 15 contains no limitation that the donor and recipient are not of the same species, and that the embryo of claim 15 is, therefore, anticipated by the embryos of Barnes *et al.*, which were produced by IVF using bovine

oocytes and bovine sperm. Applicants have amended claim 15 to clarify that the embryo comprises cytoplasm and cell membrane from a bovine oocyte and differentiated cytoplasm, differentiated cell membrane, and nucleus derived from a differentiated cell of a species other than bovine. In view of the amendment, Applicants request that the rejection be withdrawn.

The Examiner concedes that the embryo of claim 12 would, as a single cell at the time of fusion, be distinguishable from an embryo derived through IVF. The Examiner asserts that in later stages of development, the embryo of claim 12 would be indistinguishable from an embryo made by IVF, because "as the embryo develops the maternal molecules decay and the process becomes dependent on expression of genetic information, i.e., the nuclear donor. (see Introduction section of Telford *et al.*) Therefore, because development of the embryo ultimately becomes controlled by the nuclear donor, an embryo at later stages of development would be indistinguishable from that derived [by] other means. "

Applicants respectfully disagree with the Examiner's assertion that an embryo made according to claim 12 would necessarily be the same as one made by IVF. Applicants respectfully submit that the embryo of claim 12 is distinguishable from an embryo obtained by intraspecies IVF because an embryo according to claim 12 would retain bovine mitochondrial DNA and the nuclear DNA of the species other than bovine, whereas an embryo made by IVF would have mitochondrial and nuclear DNA of a single species. It is now well established that mammalian oocytes contribute mitochondrial DNA to the developing embryo and that maternally-derived mitochondrial DNA persists, and in fact, predominates, from fertilization through maturity. For example, please see the article entitled "Dolly is not quite a clone", which was published in the 31 August 1999 issue of Nature Science Update (http://www.nature.com/nsu/990902/990902-5.html), a copy of which is attached as Exhibit A. Applicants submit that an embryo of any stage of development made according to claim 12 would be distinct from an embryo obtained by intraspecific IVF because it would have bovine mitochondrial DNA and nuclear DNA from a species other than bovine, and would not, therefore, necessarily be the same as an embryo made obtained by intraspecific IVF. In view of the foregoing, Applicants respectfully request that the rejection be withdrawn.

Claims 12, 14, and 15 stand rejected as being anticipated by Gurdon (J. Cell Sci., 1986). The Examiner acknowledged that the Gurdon's survey of hybrid nuclear transplant embryos, summarized in Fig. 6, did not include embryos made using a bovine oocyte, as the claims require. The Examiner further asserts that "Gurdon summarizes the early success of

nuclear transfer in mammals (page 312, citing the work of Hoppe, Illmensee, Kelly and McGrath)."

Applicants point out that in the discussion the Examiner referenced, specifically, the second full paragraph on page 312, Gurdon discusses "the special difficulty that seems to afflict nuclear transplantation in mammals." Gurdon referenced McGrath and Solter, who were unable to repeat the results reported by Illmensee and Hoppe. In fact, Applicants submit that no one was able to repeat the "work" of Illmensee and Hoppe, which is now famous primarily because of controversy surrounding the veracity of their report, and because an international commission judged the results reported by Illmensee and Hoppe to be false.

A valid rejection under 35 U.S.C. 102(b) requires that the cited art teach every claim limitation. In his review, Gurdon does not report the existence of a trans-species nuclear transfer embryo originating in part from a recipient mammalian oocyte, nor from a recipient bovine oocyte, according to the claims as amended. Neither Gurdon nor the work cited therein teach a trans-species nuclear transfer embryo made by inducing the donor cell of a species other than bovine to undergo G_0 arrest, fusing the donor cell to an enucleated bovine ooctye to create a nuclear transfer embryo, and activating the nuclear transfer embryo. Therefore, because Gurdon does not teach all of the claim limitations, Applicants respectfully submit that claims 12, 14, and 15 are not anticipated by Gurdon and request that the rejection under 35 U.S.C. 102(b) be withdrawn.

Rejection under 35 U.S.C. 102(e)

Claims 12, 14, and 15 are rejected under 35 U.S.C. 102(e) as being anticipated by Stice *et al.* The Claims 12, 14, and 15 stand rejected as being anticipated by Stice *et al.* (WO 95/17500), on which international application ABS Global is the applicant. Examiner asserts that because claim 15 encompasses "a single cell comprising the cytoplasm from one species and the nuclear material from a second different species, the claims now clearly encompass a chimeric single cell embryo. Stice *et al.* teach nuclear transfer procedures for producing non-human chimeric animals. Specifically, nuclear transfer techniques are used to introduce the nuclear material of one species of animal into the enucleated oocyte of a recipient animal (entire disclosure and specifically claimed in claims 1-39)." The Examiner further characterized Stice *et al.* as teaching that "various combinations of species can be done."

However, Applicants have reviewed Stice *et al.*, and were unable to find any discussion of trans-species nuclear transfer. Under the Field of Invention, at page 1, lines 19-23, Stice *et al.* teach producing chimeric ungulate embryos made by introducing blastomeres

obtained from a fertilized embryo into a nuclear transfer embryo produced using an embryonic stem cell as a donor. Although "chimeric" is not expressly defined in Stice *et al.*, Stice *et al.* discusses chimeric in the context of chimeric offspring produced "by combining tetraploid mouse embryos with mouse embryonic stem cells" (see page 15, lines 1-15 of Stice *et al.*), which clearly indicates that Stice *et al.* did not use the word "chimeric" to refer to a nuclear transfer embryo made from two different species of organisms. The Examples at pages 35-40 did not disclose any species of animal, much less using two different species of animal to make a nuclear transfer embryo. The Examiner stated that the Examples show that the "oocyte is cultured for 16 hours, enucleated and donor nuclear material is transferred to the perivitelline space and fused by electrofusion." However, Stice *et al.* does not teach trans-species nuclear transfer.

Applicants direct the Examiner's attention to the steps of the methods disclosed by Stice *et al.* A comparison of the steps of the disclosed method of nuclear transfer (beginning at page 16, line 13 of Stice *et al.*) with those of the disclosed method of producing non-human chimeric embryos (beginning at page 17, line 3 of Stice *et al.*) reveals that the only difference is that the latter method includes step (vii), which involves introducing one or more blastomeres into the perivitelline space of the cultured nuclear transfer embryo [formed by steps (i)-(vi)]. Stice *et al.* does not suggest using two different species to make the nuclear transfer embryo of steps (i)-(vi). In other words, a nuclear transfer embryo made by steps (i)-(vi) is used in step (vii) to make a chimeric embryo by introducing a blastomere into the perivitelline space of the cultured nuclear transfer embryo. In Example 5, the only one of the Examples that mentions "chimeric embryos", a chimeric embryo was said to be produced by transferring "two blastomeres from an 8-16 cell stage fertilized embryo into the perivitelline space of an eight to 16-cell embryonic stem cell clone." There is no mention of using different two different species in any aspect of the method, let alone any discussion of a nuclear transfer embryo made from two different species.

Furthermore, the chimeric embryos made by embryonic transplantation or by splitting and recombining embryos, as described in Stice, would have a nucleus with chromatin from two different sources. In contrast, the nuclear transfer embryo of the present invention would have only the chromatin of the nuclear donor cell.

Because Stice *et al.* does not teach an embryo according to any of claims 12, 14, or 15, Applicants respectfully request that the rejection under 35 U.S.C. 102(e) be withdrawn.

Rejection of claims under 35 U.S.C. 103(a)

Claims 1-15 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Prather *et al.* (Biology of Reproduction, 1989, Gurdon *et al.* (J. Cell Sci. 1986), Campbell *et al.* (WO 97/07668, March, 1997), Telford *et al.* (Molecular Reproduction and Development, 1990), Dominko *et al.* (Molecular Reproduction and Development, 1997), and Stice *et al.*

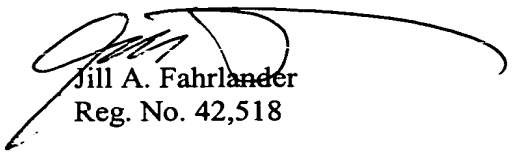
The Examiner found Applicants arguments' that the prior art does not teach trans-species nuclear transfer into an enucleated mammalian recipient oocyte to be unpersuasive and asserted that Gurdon teaches that "trans-species nuclear transfer had been attempted for a wide variety of species of animals, and in view of the teachings of the reference as a whole provides for the use of recipient mammalian oocytes." Applicants respectfully disagree with the Examiner's characterization of Gurdon. The only hybrid nuclear transplant embryos taught by Gurdon employed amphibian oocytes as recipients. As discussed above, Gurdon emphasized the "the special difficulty that seems to afflict nuclear transplantation in mammals" and does not teach or suggest trans-species nuclear transfer using an enucleated bovine oocyte as a recipient cell. As discussed above, Stice *et al.* does not demonstrate interspecies nuclear transfer. Stice *et al.* discloses producing an intraspecies nuclear transfer embryo and then forming a chimeric embryo by transferring two blastomeres from an eight to 16 cell-stage embryo into the perivitelline space of an eight to 16-cell stage embryonic stem cell clone. The resulting chimeric embryo would be distinct from the claimed embryos of the present invention, in that the former would have a nucleus with chromatin from two different sources. In contrast, the nuclear transfer embryo of the present invention would have only the chromatin of the nuclear donor cell. Whereas the embryos of the present invention are true nuclear transfer embryos and transmit the nuclear genome of only the donor cell to descendant cells, that is not true of the chimeric embryos of Stice *et al.*

Because there is no suggestion in the art to make a trans-species nuclear transfer embryo using a bovine oocyte as the recipient and a donor cell of a species other than bovine, and the art provides no reasonable expectation of success, a prima facie case of obviousness has not been established. Applicants respectfully request that the rejections under 35 U.S.C. 103(a) be withdrawn.

As the application is now in condition for allowance, Applicants request allowance of the claims. Should the Examiner feel that any other point requires consideration or that the form of the claims can be improved, the Examiner is invited to contact the undersigned at the number listed below.

No fee is believed due in connection with this submission. Please charge any fee due or credit any overpayment of fees to Deposit Account No. 50-0842.

Respectfully submitted,



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MARKED VERSION OF AMENDED CLAIMS UNDER
37 CFR § 1.121(c)(1)(ii)

All the words, phrases, or numbers added to the claims are underlined, and all words, phrases, or number removed from each such claim are enclosed in brackets ("[ ]"). Newly added text is enclosed in double dashes (--).

1. A method of producing nuclear transfer embryos from donor cells of a first species other than bovine and recipient bovine oocytes [from a second species] comprising:

inducing the donor cells to undergo G_0 arrest;

fusing said donor cell to an enucleated recipient bovine oocyte [of the second species] to create a nuclear transfer embryo[, wherein the first and second species are not the same, and wherein the second species is a mammalian species];

and activating said nuclear transfer embryo.

15. A non-human nuclear transfer embryo comprising cytoplasm and cell membrane from a bovine oocyte [first species, wherein the first species is a mammalian species,] and differentiated cytoplasm, differentiated cell membrane, and nucleus derived from a differentiated cell of a [second] species other than bovine.

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Dolly is not quite a clone

31 August 1999

HENRY GEE

Doubt has been cast before on the parentage of Dolly the famous cloned sheep – but a surprising new twist in her remarkable story comes in the latest issue of Nature Genetics[1], from Eric A. Schon of Columbia University, New York and colleagues (including researchers from the Roslin Institute and PPL Therapeutics in Scotland, where Dolly was created).

To create Dolly, a mature cell from the mammary gland of one sheep was fused with the oocyte (egg cell) from another, from which oocyte the nucleus had previously been removed. The nucleus is the repository of the cell's genetic material. The result of this fusion was a cell with the vigour and potential of an oocyte but a genetic constitution determined by the nucleus of the mammary-gland cell. This cell eventually grew into Dolly – a sheep whose nuclear DNA was cloned from a single mammary-gland cell. As far as nuclear DNA is concerned, Dolly is a true clone of the sheep that donated the mammary-gland cell.

But there's more to genetic material than the DNA in the nucleus. Floating inside the cytoplasm of the cell – the jelly-like part of the cell outside the nucleus – are bodies called mitochondria. These tiny sausage-shaped 'organelles' are responsible for generating energy, and contain genes of their own, distinct from DNA in the nucleus. Cells, therefore, contain not one but two distinct genomes – one in the nucleus, the other in the mitochondria.

As Schon and colleagues demonstrate, Dolly the sheep contains the nuclear genome of the mammary-gland cell from which she was cloned, but the mitochondria from the oocyte with which that mammary gland cell was fused. In genetic terms, she is a 'chimaera', with DNA of different origins.

Curiously, this is not quite the result the researchers were expecting. A mammalian oocyte is a huge cell containing perhaps 100,000 mitochondria. The mature mammary-gland cell that was merged with the oocyte was much smaller, containing between 2,000 and 5,000 mitochondria. This is far fewer than in an oocyte, to be sure, but the researchers

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EXHIBIT A

could find *no* mitochondria in cloned sheep that could have been derived from the mature cell. *All* came from the oocyte. How could this be?

The researchers take a clue from conventional sexual reproduction, a cell-fusion event in which a sperm cell fuses with an egg cell. It is usually thought that the sperm contributes no more than its nucleus. And so it does – eventually. Initially, sperm-derived mitochondria enter the oocyte along with the nucleus, but these mitochondria are actively destroyed, leaving only the oocyte-derived mitochondria (this explains why the inheritance of mitochondrial DNA is exclusively maternal.) The researchers speculate that, although the recipient oocyte has lost its nucleus, it still retains the capacity to wipe out invading mitochondria.

References

1. Schon, E.A. et al. Mitochondrial DNA genotypes in nuclear transfer-derived cloned sheep . ***Nature Genetics***, **23**, 90 - 93, (1999).

EXHIBIT A